SPECIFICATION

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APPARATUS AND METHOD FOR SCANNING MULTIPLE ARRAYS OF BIOLOGICAL PROBES

Cross Reference to Related Applications

The present application claims priority from U.S. Provisional Patent Applications Serial Nos. 60/242,975 and 60/242,859, both filed October 24, 2000 and both hereby incorporated by reference herein in their entireties for all purposes; and from U.S. Provisional Patent Application Serial No. 60/244,817, filed October 31, 2000.

Background of Invention

[0001] Field of the Invention: The present invention relates to optical scanning systems for examining biological material disposed on a substrate.

[0002] Related Art: Microarrays with extremely large number of probes are manufactured by methods described in U.S. Patents 5,143,854; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,445,934; 5,744,305; 5,800,992; 6,040,138; 6,040,193; 6,140,044; 6,150,147; 6,153,743; and 6,291,183; and in PCT Application PCT/US91/08693, published as WO 92/10092; all of which are hereby incorporated herein in their entireties by reference. The probes may have dimensions from about 10 microns to 100 microns. Each probe may include several million DNA molecules. After exposing the microarray to target molecules under selected test conditions, scanning devices can examine locations in the array to determine whether target molecules have interacted with probes at those locations.

[0003] Microarray technology has been used to analyze a large number of complex biochemical reactions and systems. This technology provides a massively parallel form of analysis that increases data collection per unit time, decreases the overall time required

for analysis, and uses smaller sample volumes and reagent volumes. For these and other reasons, microarray technology is well suited for genomic research. For example, microarrays have been used for monitoring the expression levels of a multiplicity of genes. See, e.g., U.S. Patent Nos. 6,040,138; 6,027,880; and 6,185,561; all of which are hereby incorporated in their entireties herein by reference for all purposes.

Summary of Invention

[0004] Systems and methods are described herein that increase throughput by enabling scanning of multiple biological chip assays (e.g., microarrays used for hybridization experiments) on a single wafer disposed in a convertible processing apparatus, thus significantly reducing the time required to prepare and present microarrays for scanning. This scanning of multiple arrays (e.g., arrays of microarrays) is sometimes referred to herein as parallel scanning in that numerous microarrays may be processed in a batch rather than one at a time. Advantageously, this parallel scanning operation may be combined with parallel processing of certain experimental stages. That is, a convertible processing apparatus is used that enables separate hybridization of sample targets to each of the microarrays, but readily converts to a single flow cell for parallel fluidic steps such as washing, staining, preserving, or removal of non-hybridized sample. While the array of microarrays remains in the convertible processing apparatus, the microarrays may be scanned in parallel, thus further streamlining the experiment. Indeed, in some implementations the fluidic steps, including hybridization reactions, may be conducted while the convertible processing apparatus is disposed on, or otherwise coupled to, a translation stage of the scanner, thereby eliminating intermediate handling steps.

[0005] It will be understood that the implementations described herein are illustrative only. For example, although optical scanning systems are described herein that detect fluorescent radiation from labeled targets that have hybridized to probes on the microarrays, other scanning and labeling systems may be used. As a further example, illustrative embodiments are described that are directed to arrays of microarrays on a wafer such as are synthesized in the manufacture of Affymetrix ® GeneChip ® arrays made by Affymetrix, Inc. of Santa Clara, California. It should be understood, however, that many other types of microarrays and other kinds of parallel assays may be used.

[0006] In accordance with one illustrative embodiment, a multi-array scanning system is

described for scanning a plurality of microarrays disposed on a substrate. The system includes a scanner apparatus that is capable of detecting emission signals from at least two of the plurality of microarrays. The scanning apparatus may be of a variety of designs such as, for example, are described in U.S. Patent Nos. 5,981,956; 6,207,960; and 6,225,635; all of which are hereby incorporated herein by reference in their entireties for all purposes. Also included is a convertible processing apparatus having one or more containing members constructed and arranged to contain the substrate and a separating member. The separating member is constructed and arranged so that, when it is disposed in a first position with respect to the containing members, the at least two microarrays are fluidically separated from each other by the separating member, and, when the separating member is disposed in a second position with respect to the containing members, the at least two microarrays are fluidically coupled with each other.

[0007] In some implementations, the scanner apparatus includes an excitation radiation source and a focusing system constructed and arranged to focus radiation from the excitation radiation source onto a selected first portion of the substrate including the at least two microarrays. Also included in these implementations is a radiation direction system constructed and arranged to scan the focused excitation radiation across the first portion of the substrate. Other elements of these implementations include a detector constructed and arranged to detect the emission signals from the first portion of the substrate in response to the focused excitation radiation, and a data acquisition system constructed and arranged to record an amount of the emission signals detected as a function of positions on the first portion of the substrate from which the emission signal was emitted.

[0008] The focusing system may include an objective lens having a ratio of scanning field diameter to focused spot diameter of greater than about 2000, and a numerical aperture greater than about 0.2. The radiation direction system may include a galvanometric mirror, angularly oscillating mirror, or a rotating polyhedral mirror, any one of which may be used for reciprocally scanning the focused excitation radiation. The focused excitation radiation may be reciprocally scanned across a second portion of the substrate including a selected one of the at least two microarrays at a rate of at least 20 image lines per second. In some implementations, the data acquisition system includes a computer having a processor and a memory. The computer receives image data representing the

detected emission signals from the scanner apparatus and stores the image data in the memory.

[0009] The computer may be programmed to execute a computer program application designed for scanner control, data acquisition, and data analysis. This program may control the focusing system and the radiation direction system so as to sequentially focus on and irradiate a first of the at least two microarrays and then sequentially focus on and irradiate one or more other of the at least two microarrays. The word sequentially in this context means that focusing and scanning are done on one of the microarrays, then focusing and scanning are done on another of the microarrays, and so on for all or any selected portion of the microarrays. A scanner user, employing a graphical user interface or other conventional technique, may specify the order of this sequential scanning and/or those particular microarrays of the array of microarrays that are to be scanned. In various implementations, the convertible processing apparatus is coupled to a translation stage. A translation stage controller moves the translation stage, under the direction of the computer, in coordination with the focusing system and radiation direction system. For example, the translation stage controller may move the translation stage in an X direction, a Y direction, or both so as to sequentially position each of the at least two microarrays for irradiation. The translation stage controller may also move the translation stage in a Z direction orthogonal to a plane of the X and Y directions so as to sequentially position each of the at least two microarrays for focusing.

[0010] In accordance with other embodiments, a method is described for scanning a plurality of microarrays (e.g., an array of microarrays) disposed on a substrate. The preferred method includes (1) fluidically separating at least two of the plurality of microarrays from each other; (2) contacting the at least two microarrays with one or more target solutions while the at least two microarrays are fluidically separated; (3) retaining the fluidic separation of the at least two microarrays for a first period of time sufficient for hybridization reactions, if any, to occur between the target solutions and the at least two microarrays; (4) fluidically coupling the at least two microarrays after the first period has elapsed; (5) performing one or more parallel fluidic processes on the at least two microarrays based, at least in part, on the fluidic coupling; and (6) detecting emission signals from at least two of the plurality of microarrays.

[0011] In some additional preferred implementations of these methods, step (6) further comprises the steps of (a) providing an excitation radiation source; (b) focusing radiation from the excitation radiation source onto a selected first portion of the substrate including the at least two microarrays; (c) scanning the focused excitation radiation across at least the first portion of the plurality of microarrays; (d) detecting the emission signals from the first portion in response to the focused excitation radiation; and (e) recording an amount of the emission signals detected as a function of positions on the first portion from which the emission signal was emitted. Step (6) (c) may include the step of reciprocally scanning across a selected one of the at least two microarrays at a rate of at least 20 image lines per second. Step (6) (e) may include the steps of receiving image data representing the detected emission signals and storing the image data in a computer memory.

[0012] In yet further preferred implementations, step (6) may include (a) providing an excitation radiation source; (b) focusing radiation from the excitation radiation source onto a selected first portion of a substrate including a first of the at least two microarrays; (c) scanning the focused excitation radiation across the first portion of the substrate; (d) detecting the emission signals from the first portion of the substrate in response to the focused excitation radiation; (e) recording an amount of the emission signals detected as a function of positions on the first portion of the substrate from which the emission signal was emitted; (f) moving the substrate to enable focusing of radiation from the excitation radiation source onto a selected second region of the substrate including a second of the at least two microarrays; (g) scanning the focused excitation radiation across the second portion of the substrate; (h) detecting the emission signals from the second portion of the substrate in response to the focused excitation radiation; and (i) recording an amount of the emission signals detected as a function of positions on the second portion of the substrate from which the emission signal was emitted.

[0013] In accordance with a further embodiment, a method is described for analyzing nucleic acids using a plurality of nucleic acid microarrays. The method includes (1) preparing a cell sample having nucleic acids; (2) contacting the sample with an apparatus that comprises one or more containing members constructed and arranged to contain the plurality of nucleic acid microarrays, and a separating member constructed and arranged

so that, when the separating member is disposed in a first position with respect to the containing members, at least two of the plurality of nucleic acid microarrays are fluidically separated from each other by the separating member, and when the separating member is disposed in a second position with respect to the containing members, the at least two microarrays are fluidically coupled with each other; (3) providing an excitation radiation source; (4) focusing radiation from the excitation radiation source onto one or more of the plurality of microarrays; (5) scanning the focused excitation radiation across the one or more microarrays; and (6) detecting the emission signals from the one or more microarrays in response to the focused excitation radiation. The method may also include the step of (7) recording an amount of the emission signals detected as a function of positions from which the emission signal was emitted.

[0014] In another embodiment, a method is described for analyzing nucleic acids using a plurality of nucleic acid microarrays. The method includes (1) preparing a cell sample having nucleic acids; (2) contacting the sample with an apparatus that comprises one or more containing members constructed and arranged to contain the plurality of nucleic acid microarrays; (3) providing an excitation radiation source; (4) focusing radiation from the excitation radiation source onto one or more of the plurality of microarrays; (5) scanning the focused excitation radiation across the one or more microarrays; and (6) detecting the emission signals from the one or more microarrays in response to the focused excitation radiation. In these embodiments, the apparatus may further comprise a separating member constructed and arranged so that, when the separating member is disposed in a first position with respect to the containing members, at least two of the plurality of nucleic acid microarrays are fluidically separated from each other by the separating member, and when the separating member is disposed in a second position with respect to the containing members, the at least two microarrays are fluidically coupled with each other.

[0015] In accordance with yet other embodiments, a method is described for scanning a plurality of microarrays, comprising the steps of: (1) contacting at least two microarrays of the plurality of microarrays with one or more target solutions; (2) performing one or more parallel fluidic processes on the at least two microarrays; and (3) detecting emission signals from the at least two microarrays.

[0016] The above embodiments and implementations are not necessarily inclusive or exclusive of each other and may be combined in any manner that is non-conflicting and otherwise possible, whether they be presented in association with a same, or a different, aspect of the invention. The description of one embodiment or implementation is not intended to be limiting with respect to other embodiments or implementations. Also, any one or more function, step, operation, or technique described elsewhere in this specification may, in alternative embodiments or implementations, be combined with any one or more function, step, operation, or technique described in the summary. Thus, the above embodiments and implementations are illustrative rather than limiting.

Brief Description of Drawings

[0017] Figure 1 is a diagram of stacked segments of a processing array assembly in accordance with the present invention;

[0018] Figure 2 is a front view of a grid plate of a processing array assembly in accordance with the present invention;

[0019] Figure 3 is side view of a grid plate of a processing array assembly in accordance with the present invention;

[0020] Figure 4 is a cross section of the grid plate shown in Figure 2 taken along line A-A;

[0021] Figure 5 is a bottom view of a base plate of a processing array assembly in accordance with the present invention;

[0022] Figure 6 is a top view of a base plate of a processing array assembly in accordance with the present invention;

[0023] Figure 7 is a side view of a base plate of a processing array assembly in accordance with the present invention;

[0024] Figure 8 is a cross section view of region A of the grid plate as shown in Figure 7;

[0025] Figure 9 is a top view of a top plate of a processing array assembly in accordance with the present invention;

[0026] Figure 10 is a cross section of the top plate shown in Figure 9 taken along line A-A;

[0027] Figure 11 is a side view of the top plate shown in Figure 9 taken along line B-B";

[0028] Figure 12 is a top view of a top support plate of a processing array assembly in accordance with the present invention;

[0029] Figure 13 is a side view of the top support plate shown in Figure 12 taken along line at A-A";

[0030] Figure 14 is a top view of a bottom support plate and grid seal of a processing array assembly in accordance with the present invention;

[0031] Figure 15 is a cross section of the bottom support plate and grid seal of in Figure 14 taken along line A-A";

[0032] Figure 16 is a side view of the bottom support plate and grid seal in accordance with the present invention;

[0033] Figure 17 is an enlargement of region C of the bottom support plate and grid seal shown in Figure 14;

[0034] Figure 18 is an enlargement of region B of the cross section of the bottom support plate and grid seal shown in Figure 15;

[0035] Figure 19 is a top perspective view of an assembled processing array assembly in accordance with the present invention;

[0036] Figure 20 is a cross section view of the assembled processing array assembly in accordance with the present invention;

[0037] Figure 21 is an enlargement of region A of the apparatus shown in Figure 20;

[0038] Figure 22 is a top view of a view plate of a processing array assembly in accordance with the present invention;

[0039] Figure 23 is a cross section view of the view plate shown in Figure 22 taken along line A-A;

[0040] Figure 24 is an enlargement of region A of view plate as shown in Figure 23;

[0041] Figure 25 is a side view of a view plate of a processing array assembly in accordance

with the present invention;

[0042] Figure 26 is a cross sectional view of an edge detail of a processing array assembly in accordance with the present invention; and

[0043] Figure 27 is a functional block diagram of one embodiment of a multi-array scanning system for scanning an array of microarrays disposed within a processing array assembly such as that of Figure 1-26.

Detailed Description

[0044] A multi-array scanning system is now described in accordance with one illustrative embodiment of the present invention. As shown in Figure 27 and described in greater detail below in relation to that figure, the scanning system includes a scanner apparatus that detects emission signals from microarrays in an array of microarrays. The scanning system also includes a convertible processing apparatus that, as noted, enables parallel processing with respect to some aspects of hybridization experiments carried out on the array of microarrays. One illustrative implementation of the convertible processing apparatus, referred to as apparatus 5, is now described in relation to Figures 1-26.

[0045] Convertible Processing Apparatus 5Methods previously have been described for carrying out fluidic operations with respect to multiple microarray assays in U.S. Patent Nos. 5,545,531 and 5,874,219, both of which are hereby incorporated herein by reference in their entireties for all purposes. Those previously described multiple-processing methods provide significant advantages over traditional approaches in which microarrays are separately processed. Illustrative apparatus 5 provides further advantages by, among other things, combining the capabilities for separate hybridization of multiple arrays in fluidically separated hybridization chambers with parallel processing of those arrays in a single fluidic chamber during certain processing stages.

[0046] Apparatus 5 is suitable for processing a plurality of microarrays disposed on a substrate. Illustrative apparatus 5 includes one or more containing members that contain the substrate. The apparatus also includes a separating member constructed and arranged so that, when the separating member is disposed in a first position with respect to the containing members, at least two of the plurality of microarrays are fluidically separated from each other by the separating member. Moreover, when the separating

member is disposed in a second position with respect to the containing members, the at least two microarrays are fluidically coupled with each other. Thus, in some implementations such as illustrated by apparatus 5, separate microarray hybridization chambers are converted to a single fluidic chamber by moving the separating member.

[0047] In these contexts, the term fluidically separated and grammatical variants are used broadly to mean, for example, that a fluid disposed so as to interact with one microarray does not interact with another microarray. Similarly, the term fluidically coupled and grammatical variants are used broadly to mean, for example, that a fluid may interact with more than one microarray by, for instance, flowing over both microarrays in the same fluidic cell or chamber. The fluid may thus be referred to herein as operating in parallel with the two or more microarrays, or being involved in parallel fluidic processes, even though it is not necessary in all implementations that the fluid contact each of the microarrays evenly, or at the same instant.

[0048] In some implementations, the one or more containing members include a first segment and a second segment in contact with the first segment, wherein the substrate is disposed between the first and second segments. The separating member may be disposed between the first and second segments when the separating member is in the first position, and may be disposed apart from the first and second segments when the separating member is in the second position. The substrate may be retained in place by the first and second segments. The first segment may include a central frame, which may have an inlet port for receiving fluids and an outlet port for expelling fluids. The separating member may include one or more walls constructed and arranged to fluidically separate the at least two microarrays when the separating member is disposed in the first position.

[0049] The separating member may include a grid plate that has grid elements determined by walls. For example, a grid element may be a chamber made up of a group of the walls and having one surface made up of a portion of the grid plate lying within the group of walls. In these implementations, each of the at least two microarrays is fluidically separated from each of the other at least two microarrays by a grid element (e.g., grid chamber) when the separating member is disposed in the first position. Each of the at least two microarrays is fluidically coupled with the other at least two microarrays when

the separating member is disposed in the second position.

[0050] In a preferred embodiment, the microarrays may be synthesized probe arrays, wherein the probes comprise oligonucleotides. In some other preferred implementations, the oligonucleotides may have been synthesized to the microarrays based, at least in part, on photolithographic methods such as described, for example, in U.S. Patent No. 5,143,854, incorporated by reference above. In particular, the microarrays may be disposed on a contiguous surface of the substrate comprising what is referred to for convenience herein as a photolithographic wafer. This term refers in this context to wafers of multiple arrays produced, at least in part, by photolithographic processes. The production of microarrays on wafers is described, for example, in U.S. Patent Application Serial No. 09/824,931, filed April 3, 2001, which is hereby incorporated by reference herein in its entirety for all purposes. In other implementations, however, wafers or other contiguous substrates may be employed on which microarrays have been synthesized or deposited using any of a variety of known techniques (such as electrical, mechanical, ink jet, or the like), many of which do not include photolithographic processes.

[0051] Apparatus 5 may be referred to as a multi-segmented processing apparatus. The apparatus includes a number of stacked segments allowing for access to different segments within the stack, as desired during use. In the illustrated implementation, the apparatus includes various segments that, for the sake of convenience only, are called top segment, grid segment, sample segment, base segment and view segment. There may also be seals arranged between two or more of the stacked segments as needed. It should be appreciated that the names of the segments are chosen for convenience, and the apparatus may be oriented, for example, with the "top" segment on the lower side, or the "base" segment oriented on an upper side, or the apparatus disposed in a generally vertical as opposed to horizontal orientation. Accordingly, "top" is used in this description to characterize an orientation that is on the opposite side of a "base" or "bottom" and should not be taken as a characterization of upper versus lower orientations.

[0052]

In the illustrated implementation, a grid segment is constructed of an array or grid of generally impermeable walls, forming the four edges of an individual sample chamber. The term generally impermeable is used broadly in this context to mean that chambers

are fluidically separated from each other. A grid seal is connected to the grid segment and is designed to uniformly and efficiently seal with very low contact pressure. The grid segment, with its accompanying grid seal is mounted to the base segment. The base segment includes a base plate and a bottom support plate. The grid segment is mounted on top of the base plate. An O-ring is also positioned in the base plate. A sample segment such as a glass plate is positioned atop the grid seal, i.e. on the opposite side of the grid seal from the grid segment. In such an arrangement, the sandwich of the glass plate, grid seal and grid plate creates an array of liquid-tight (i.e., fluidically separated) chambers. Preferably, the seal does not contact any of the microarrays on a sample segment, thus allowing for precise alignment of the glass plate and the grid seal. Advantageously, this increased precision in aligning the glass plate with the grid seal enables more samples to be positioned on each plate.

[0053] In the illustrated implementation, the top segment is comprised of a top support plate and a top plate. The outer surface of the top segment is continuous, stepless, and smooth with a minimal gap between the top support plate and the top plate. It is preferred that such a generally smooth surface be used to reduce the likelihood of contamination, although it should be appreciated that other surface provides can be used, such as undulating, curved, etc. The top segment is positioned on one side (i.e. a "top" side) of the glass plate. On the other side of the glass plate is arranged, preferably in this order, the grid plate, the o-ring, and the base segment. All these segments can be fastened together such that metal to metal contact is achieved between the top segment and the base segment. Thus the outer dimensions of the glass plate and grid plate preferably are selected to be smaller than those of the top and bottom segments so as to allow them to fit within the perimeters of the top and bottom segments. By arranging the top segment and base segment to contact one another allows for seal loading independent of fastener torque such that seal loading is consistent and independent of any fastener variation or fastener torque. Accordingly, operator inconsistency in how tight segments are fastened with one another can be reduced or eliminated.

[0054] As noted above, the assembled multi-segmented array may be oriented in any desired direction. The apparatus optionally may be turned over by an operator, so the glass plate forms the bottom of individual sample chamber(s) and the grid walls of the grid plate forms the walls between individual sample chambers. A unique sample (e.g.,

target solution) may be placed in each individual sample chamber within the array. Once sealed, the grid creates a liquid tight seal between each individual chamber within the array. Advantageously, the sealed array may undergo multiple process steps, such as hybridization, shaking, or incubation, for the duration of the reaction.

[0055] It is an advantage of some embodiments that the multi-segmented array apparatus enables imaging and detecting within the apparatus itself. Pre-detection processing may also occur within the apparatus. In operation, the bottom support optionally may be removed and a view plate installed, thus creating a flow cell chamber in which pre-detection processing may occur directly on the samples on the glass plate.

[0056] It is a further advantage of some embodiments that manipulations are performed without the need to transfer to an entirely different processing device. Costs for processing reagents, test samples and cleanup may be reduced. Moreover, eliminating the need to transfer samples to a separate device between detection pre-processing steps and the detecting step enables smaller, low volume amounts of samples to be used. As another added advantage in some implementations, because the samples are not removed from the original apparatus, multiple detecting steps may be practiced on a single array by pre-processing for another detection step and moving the apparatus to another detecting device.

[0057] In one aspect, the segmented processing array includes a plurality of segments arranged in stacked relation, the segmented processing array comprising a first segment, a second segment in contact with the first segment, and a processing array positioned between the first segment and the second segment, and retained in place by the first and second segments. In another implementation, the microarray processing system includes multiple processing chambers, the processing chambers comprising: a plate member between the first and second segment, a grid segment containing an array of chamber walls, and a bottom support segment, the grid segment positioned between the bottom support segment and the plate member forming a first surface of the processing chambers and the plate member forming a second surface of the processing chambers.

[0058] In various implementations, the processing system is used to process arrays of microarrays on a substrate, such as, for example, a wafer. One preferred type of microarray produced in groups on a wafer and then, conventionally, diced to allow

individual packaging is the Affymetrix ® GeneChip ® probe array available from Affymetrix, Inc. of Santa Clara, California. The GeneChip ® probe array is synthesized using photolithographic methods, as noted above. Various other techniques for synthesizing microarrays (i.e., producing probes in situ) are available. While illustrated implementations may be described herein with respect to synthesized microarrays, and the GeneChip ® type array in particular, it should be noted that the apparatuses and methods described herein may be applied with respect to many other types of probe arrays and, more generally, with respect to numerous parallel biological assays produced in accordance with other conventional technologies and/or produced in accordance with techniques that may be developed in the future. For example, aspects of the apparatuses and methods described herein may, in some implementations, be applied to parallel assays of nucleic acids, PCR products generated from cDNA clones, proteins, antibodies, or many other biological materials. These materials may be disposed on slides, on substrates employed for GeneChip ® arrays, or on beads, optical fibers, or other substrates, supports, or media (all or any of which may hereafter generally and collectively be referred to as substrates). See also, U.S. Pat. No. 5,143,854 for additional substrates. Moreover, with respect to some implementations in which the context so indicates or allows, the probes need not be immobilized in or on a substrate, and, if immobilized, need not be disposed in regular patterns or arrays. For convenience, the terms probe array or microarray will generally be used broadly hereafter to refer to all of these types of arrays and parallel biological assays.

[0059]

A microarray made by depositing or positioning pre-synthesized or pre-selected probes on a substrate, or by depositing/positioning techniques that may be developed in the future, may be referred to herein as a spotted array. Typically, but not necessarily, spotted arrays are commercially fabricated on microscope slides. These arrays often consist of liquid spots containing biological material of potentially varying compositions and concentrations. For instance, a spot in the array may include a few strands of short polymers, such as oligonucleotides in a water solution, or it may include a high concentration of long strands of polymers, such as complex proteins. The Affymetrix ® 417 ™ and 427 ™ Arrayers are devices that deposit densely packed arrays of biological material on a microscope slide in accordance with these techniques. Aspects of these, and other, spot arrayers are described in U.S. Patents Nos. 6,121,048, 6,040,193 and

6,136,269, in PCT Applications Nos. PCT/US99/00730 (International Publication Number WO99/36760) and PCT/US 01/04285, in U.S. Patent Applications Serial Nos. 09/122,216, 09/501,099, and 09/862,177, and in U.S. Provisional Patent Application Serial No. 60/288,403, all of which are hereby incorporated by reference in their entireties for all purposes. Other techniques for depositing or positioning biological probes on a substrate, *i.e.* , creating spotted arrays, also exist. For example, U.S. Patent No. 6,040,193 to Winkler, et al. is directed to processes for dispensing drops of biological material. The "193 patent, and U.S. Patent No. 5,885,837 to Winkler, also describe separating reactive regions of a substrate from each other by inert regions and spotting on the reactive regions. The "193 and "837 patents are hereby incorporated by reference in their entireties. Other techniques for producing spotted arrays are based on ejecting jets of biological material. Some implementations of the jetting technique use devices such as syringes or piezo electric pumps to propel the biological material.

[0060] Synthesized and spotted microarrays typically are used in conjunction with tagged biological samples such as cells, proteins, genes or EST"s, other DNA sequences, or other biological elements. These samples, sometimes referred to herein as targets, typically are processed so that they are spatially associated with certain probes in the probe array. In one non-limiting implementation, for example, one or more chemically tagged biological samples, i.e., the targets, are distributed over the probe array. Some targets hybridize with at least partially complementary probes and remain at the probe locations, while non-hybridized targets are washed away. These hybridized targets, with their tags or labels, are thus spatially associated with the targets" complementary probes. The associated probe and target may sometimes be referred to as a probe-target pair. Detection of these pairs can serve a variety of purposes, such as to determine whether a target nucleic acid has a nucleotide sequence identical to or different from a specific reference sequence (see, for example, U.S. Patent No. 5,837,832). Other uses include gene expression monitoring and evaluation (see, e.g., U.S. Patent No. 5,800,992; U.S. Patent No. 6,040,138; and International App. No. PCT/US98/15151, published as WO99/05323), genotyping (U.S. Patent No. 5,856,092), or other detection of nucleic acids. The "832, "992, "138, and "092 patents, and publication WO99/05323, are hereby incorporated by reference herein in their entirety for all purposes.

[0061] To ensure proper interpretation of the term probe as used herein, it is noted that

contradictory conventions exist in the relevant literature. The word probe is used in some contexts to refer not to the biological material that is synthesized or deposited on a substrate, as described above, but to what has been referred to herein as the target. To avoid confusion, the term probe is used herein to refer to the elements synthesized or deposited on a substrate to respectively create synthesized or spotted microarrays.

[0062] Figure 1 is an exploded view of a particular embodiment of an apparatus for processing multiple microarrays, referred to as multiple-array processing apparatus 5. An assembled version of this embodiment is illustrated in Figures 19-21. In accordance with the illustrated implementation, a top segment 7 is composed of a top support plate 10 and a top plate 20. A base segment 8 includes a base plate 50 and a bottom support plate 60. As shown in Figures 2-4, grid plate 40 creates walls of multiple chambers 310, each one of which may be considered the walls of a sample area enclosing a microarray. In particular, walls 320 extend from the surface to form a pattern of through holes of grid plate 40. As noted, it is preferred that the walls 320 be relatively impermeable to any fluid that may be stored in chamber 310, so as to retain the fluid within the chambers 310 and avoid cross-contamination among chambers 310. In this embodiment, walls 320 extend generally perpendicularly to the surfaces 327, and thus may be referred to for convenience as vertically oriented. As noted, however, the apparatus in use may be disposed in any orientation, and thus terms such as vertical are used for illustrative purposes only.

[0063] Grid seal 30 also assists in the formation of a sealed chamber and in avoidance of cross contamination. It is positioned preferably on top surfaces 327 of the vertical walls 320 within a groove 380 formed in the top surfaces 327. Grid seal 30 preferably is formed from a compliant, flexible, inert material, for example silicone, although any suitable sealing material may be used. Any cross sectional profile may be selected for grid seal 370 so that a uniform seal can be achieved, preferably having a low contact pressure, for example a substantially tubular or u-shaped profile may be selected. The groove 380 in grid plate 40 is selected to retain grid seal 30 of the selected profile with friction contact, also promoting low insertion forces. Alternatively, an adhesive or other mechanical retaining force may be used to retain grid seal 30 in place. Preferably, the grid segment 40 is machined out of anodized aluminum, and coated with Teflon. However, it will be appreciated that grid plate 40 may be constructed out of other

materials which provide a liquid tight seal that is resistant to bacterial infection or phage contamination.

[0064] In this embodiment, the grid segment 40 is constructed as a grid plate, and these terms are used interchangeably. However, it should be appreciated that other configurations that accommodate multiple arrays of plate-based samples may also be used. Similarly, the grid seal 30 may be molded out of other flexible, inert, and liquid tight materials.

[0065] Referring to Figure 2, grid plate 40 creates an array 360 of individual sample chambers 310. While array 360 is depicted as a 7 x 7 array in Figure 2, other sizes of arrays may be configured, such as 12 x 12, 16 x 16, 24 x 24, 32 x 32, 64 x 64, 96 x 96, 24 x 32, 12 x 24 and so on. Walls 320 separate each individual sample chamber 310 by creating four walls. Alternatively, individual sample chambers 310 may be composed of 3 or 5 or any number of sides using walls 320.

[0066] In the illustrated embodiment, an outer edge 340 of grid plate 40 contains mating groove 380, which positions grid seal 30. Fasteners 395 such as screws may be used in order to fasten and aligned this grid segment 40 with other segments comprising this apparatus, through apertures, such as the illustrated apertures 390. Any form of fastener may be used that provides sufficient strength for retaining the structural integrity of stacked array 5, such as for example, guide pins, rivets, nails, nut/bolt combinations, Velcro®, and so on. Preferably a releasable fastener is used, such as a screw or nut/bolt combination, although permanent type fasteners may be desired in some applications, such as adhesives. In the illustrated example fastener(s) 395 connect grid plate 40 in to a bottom support plate 60 via aperture(s) 390.

[0067] Figure 3 illustrates a cross section of a preferred embodiment of grid segment 40. Apertures 390 are positioned at desired locations, and assist in aligning connecting segments, thus preserving the integrity of each individual sample chamber 310. Other aligning features may be used to assist in aligning segments. For example, mating grooves 380 are positioned on either end of grid plate 40 on the outer edge 340. The chambers 310 of array 360, including respective walls 320 extend from the are raised vertically in relation to frame 330. Atop each impermeable wall 320 rests grid seal 30, preferably with very low contact pressure. Preferably, grid seal 30 creates a liquid tight

seal between the impermeable wall 320 and plate member 25. In this manner, each individual chamber 310 in array 360 is isolated chemically from each other individual sample chamber 310.

[0068] Figure 4 illustrates mating groove 380 embedded in top surfaces 327 of each impermeable wall 320 allowing for positioning and placement of grid seal 30. It will be appreciated that groove 380 may be any other placement configuration that allows the grid seal 30 to seal each individual chamber 310 and the corresponding array 360 uniformly, preferably resulting in very low contact pressure between the impermeable wall 320 and grid seal 30.

[0069] Grid segment 40 in this embodiment is a grid plate and has a precision machined surface which mates to a precision machined face on base segment 50. In this example, base segment 50 is a base plate. The mounting face for grid plate 40 is on the A side of the base segment as shown in Figure 1. The base segment is machined out of 6061 aluminum and hard coat anodized. It will be appreciated that other fasteners such as magnets, adhesives, or other connecting devices known in the art may be used to connect segments together.

[0070] As assembled, the underside 328 of grid segment 40 is connected adjacent a base plate 50. Figures 5-8 illustrate the placement of grid plate 40 and base segment 50 together in assembled relation. As can be seen in these illustrations, apertures 510 serve to provide receive a fastener(s) to fasten the base plate 50 to top plate 20 and/or top support plate 10 and/or bottom support 60 via corresponding apertures in those elements. Any form of fastening arrangement may be used as described more fully above, although it is preferred that an aperture/mechanical fastener be used.

[0071] O-ring seal 45 is placed within base plate 50 preferably within receiving groove 570 although any arrangement to sufficiently mount the O-ring 45 on the base plate 50 and maintain a fluid-tight seal when assembled may be used. In assembled orientation, grid plate 40 is positioned on the side of seal 45 opposite the groove 570 and base plate 50. O-ring seal 45 is positioned in groove 570 in order to position and retain O-ring seal 45 with friction. At this point, O-ring seal 45 and grid seal 30 are facing the B side of base plate 50, as shown in Figure 1. Pipe fitting 520 serve to introduce liquids and gasses into array 360 as desired. While this embodiment shows two pipe fittings 520, more or fewer

pipe fittings may be used as desired. Apertures 540 may be used to employ removable guide pins to align a top segment 100 or any other segment with a bottom segment, such as the base plate 50. Apertures 300 are positioned in the four corners of base plate 50 and serve to connect base plate 50 with a top segment 100.

[0072] A plate member 25 is place in top of grid seal 30, forming an enclosure with the walls 320 and bottom support 60. Preferably plate member 25 is a transparent material, such as glass, although any material that can form an enclosure with grid seal 30 can be used. For example a plastic can be used as well. The plate member 25 preferably has microarrays positioned on discrete locations on its surface. These microarrays preferably are aligned with grid seal 30 and walls 320 such that the seal 30 does not contact any of the microarrays on glass plate 25 and preferably fluidically separates them from one another when the assembly 700 is fully assembled. Thus sample chambers 310 are formed with plate member 25 as one surface of individual sample chambers 310 and with walls 320 and bottom support 60 forming the other sides of the chambers 310.

[0073] Referring now to Figures 9-11, top plate 20 contains apertures 300 that preferably align with corresponding apertures in one or more of the other segments of apparatus 5. For example apertures 300 optionally may align with apertures 520 on base plate 50 and are used for positioning removable fasteners, preferably guide pins, that assist in alignment between top segment 100 and base plate 50. Apertures 600 are positioned on each side of top plate 20 and fasteners members connect top plate 20 to top support plate 10 to form top segment 100. Wedge 610 forms a rectangular frame surrounding the glass plate 25. Any form of fastener may be used, such as already discussed above.

[0074] In the embodiment shown in Figures 10 and 11, wedge 610 forms an angle with the bottom surface of top plate 10. In the illustrated embodiment an angle of 149 degrees is provided although it readily will be appreciated that other angles may be selected as well. Figure 10 shows a cross section of one embodiment of top plate 10 from Figure 9. In this illustration, guide pins placed in apertures 540 are placed on opposite sides of top plate 10. Region 550 is an open area in which top support plate 10 fits. Figure 11 is a side view of edge B as shown in Figure 9, and illustrates apertures 300 and 600, wedge 610, apertures 600 on another edge of top plate 20, removable guide pin 540, and symmetrically matched elements 600, 610, 300, and 600 approaching edge B.

[0075] Figures 12 and 13 show one embodiment of top support plate 10. Top support plate 10 fits adjacent to top plate 20 and is fastened via fastening members (such as already described above) connected through apertures 300 and 600 located in top plate 20 and top support plate 10. Wedge 710, forms a similar rectangular frame to top plate 20 and wedge 710 fits snugly inside of wedge 610. Of course it is preferred wedges 710 and 610 be at similar or identical angles so as to mate snugly. Similarly, removable guide pin apertures 540 also align with their corresponding elements in Figures 9-11 on top plate 20. Once top plate 20 and top support plate 10 are aligned, wedge 710 fits snugly against wedge 610 providing a resulting bottom face 720 such that a continuous, stepless, smooth surface exists across the entire top segment with minimal gap between top plate 20 and top support plate 10. Removable guide pins 540 and apertures 300 and 600 assist in aligning the two plates 10 and 20 comprising top segment 100. It will be appreciated that apertures 300, 600, and 540 may be configured in any fashion and shape known in the art such that their respective segments are aligned and fastened. For example, the type of connecting members, such as a magnet, may dictate the shape, design, or position of elements 300, 600, or 540.

[0076] As assembled, top segment 100 is aligned with the base plate, with plate member 25 therebetween. Removable guide pins 540 that may be inserted from top segment 100 through like apertures on base plate 50 and facilitate alignment and to retain the stack together. In the assembly process, once top segment 100 rests against plate member 25, fastening members such as screws may be inserted into apertures 300 through base plate 50 such that the bottom surface 720 of top segment 100 is secured against top mounting face 560 of base plate 50, thereby creating a tight seal through contact of bottom surface 720 and face 560. The features of base plate 50, grid plate 40, O-ring seal 45, and grid seal 30 are designed such that grid seal 30 and O-ring 45 are aligned when metal-to-metal contact is achieved between top segment 100, base plate 50, and grid plate 40. Once sealed, the entire apparatus can now be transported, tested, processed, or detected. For example, the apparatus may be disposed on, or otherwise coupled to, a translation stage of a scanner such as stage 2774 as shown in Figure 27. Also, some or all of the preceding and following processes could be implemented while the apparatus is coupled to the translation stage so that transportation is not required.

[0077] Once base plate 50 is attached to top segment 100, the entire assembly 70 as shown

in Figures 1 and 19 can now be flipped over if desired such that the A side is face up. Test sample may now be introduced into one or more of the now formed sample chambers 310. Samples may be introduced singly, serially, in parallel, or by any other means known in the art. Likewise, samples may be introduced manually or robotically. Once sealed, the individual test chambers 310 preferably are chemically isolated from one another, minimizing or eliminating cross contamination between chambers 310. By forming such discrete chambers, multiple tests can be performed in a single apparatus 5.

[0078] In operation, once samples have been introduced into chambers 310 such as via placing them on plate member 25, the array 360 of sample chambers 310 may be sealed simultaneously by fastening bottom support 60 to base plate 50 such as for example via apertures 300 and attendant fastening devices 395.

[0079] Figures 14-18 illustrate an embodiment of a bottom support 60. In order to make each individual sample chamber 310 fluid-tight, a grid seal 30 preferably is installed into bottom support 60 as shown in Figures 14-18. Exemplary structures for grid seals and mounting arrangements for grid seals already have been discussed above, and apply equally well to the sealing arrangement used in bottom support 60. Grid seal 30 is positioned in mating grooves 380, preferably providing a seal with relatively low contact pressure. Bottom support 60, including grid seal 30, is aligned using apertures 300 or other alignment members such that grid seal 30 will effectively seal each individual sample chamber 310 in the array of sample chambers 360 with surface 750 creating a cover for chambers 310, thereby preventing contamination and evaporation. A metal-to-metal contact is achieved between the top surface 760 of bottom support 60 and the bottom surface 290 of grid plate 40. Once sealed, the entire apparatus can now be transported, tested, or otherwise processed.

[0080] Figures 19-21 show the assembled assembly 700. As can be seen in Figure 19, top support plate 10 top plate 20, comprising top segment 100, and base plate 50 are aligned using apertures 300 and removable guide pins 540 or other fasteners. Fasteners also may be connected through apertures 600 to connect and align top support plate 10 with top plate 20 to form top segment 100. Metal-to metal contact is achieved between the surfaces of top support plate 10 and top plate 20, as well as between the surfaces between top plate 20 and base plate 50, in this implementation.

[0081] Figure 20 shows a cross section of assembled apparatus 700. From this view, one can see the bottom mounting surface 730 of top support plate 10 is wedged against the top surface 550 of top plate 20 wherein wedges 710 and 610 also preferably fit snugly. Bottom surface 720 of top support plate 10 directly contacts plate member 25. Plate member 25 contacts grid seal 30 which is embedded in grooves 380 in grid plate 40. Grid seal 30 rests directly on top of walls 320, such as on surface 327. Walls 320 form the walls of individual sample chambers 310 of which plate member 25 forms one surface as well. Grid seal 30 embedded in bottom support plate 60 rest atop of grid plate 40 and forms another surface of chambers 310. Figure 21 shows a detailed view corresponding to Figure 20.

[0082] In one embodiment, the samples may be processed to prepare the samples for detecting, typically an imaging process. These pre-detection processes usually include removing the samples and then flowing liquids or gases across the samples to wash, stabilize, stain, or otherwise prepare the sample. In a preferred embodiment, bottom support 60 may be removed from the assembly 700 and the samples within each individual sample chamber 310 removed and recovered via a singular or multiple tip pipettor. Another liquid can be introduced into each individual chamber 310 if desired, in order to prevent chamber 310 from drying out before further processes, such as flow processes, are started. For example, the following flow cell processes are preferably administered collectively to the array of sample chambers 360 in order to minimize variation and simplify processes. Optionally, however these processes may be performed individually on each individual sample chamber 310.

[0083] A single flow cell chamber or cavity 830 may be created by replacing grid plate 40 from base plate 50 with view plate 800, as shown in Figures 22-26. In view plate 800, O-ring seal 820 is positioned in O-ring groove 810 such that a low volume, sealed chamber is created across the array of sample chambers 360. Apertures 390 fasten and align view plate 800 with like apertures in base plate 50. In one embodiment, base plate 50 contains pipe fittings 520 such that when view plate 800 is installed, liquids or gases may be passed over all sample chambers in array 360 uniformly and quiescently. Flow cell chamber 830 may be optionally filled with liquid or gas and then sealed with valves on base plate pipe fittings 520. In a preferred embodiment, liquids or gases may be introduced via fittings 520 while apparatus is positioned directly on a detecting device,

thus offering a controlled environment directly within the each sample chamber 310 during detection.

[0084] In another preferred embodiment, top support plate 10 may also be removed such that plate member 25 is visible from side B as seen in Figure 26. Figure 26 shows one embodiment in which view plate 800 has replaced grid plate 40 in base plate 50. As can be seen, top plate 20 is aligned with base plate 50 via apertures 300, fastened with screws. O-ring seal 45 helps retain the positioning of view plate 800 adjacent to base plate 50. O-ring 820 creates flow chamber 830 between glass plate 25 and the top surface 840 of view plate 800. Once top support plate 10 is removed, detection processes such as spectrophotometry or other optical methods may be initiated through glass plate 25, as described below in greater detail with respect to illustrative multi-array scanning system 2700. Practitioners in the art will understand that other detection methods known in the art may be used in accordance with the present invention.

[0085] In one specific experimental implementation of the preceding embodiment, the assembly is a stacked, integrated device that can accommodate a 12.5 cm x 12.5 cm glass sample segment containing 49 oligonucleotide (e.g., GeneChip ® -type) microarrays arranged as a 7x7 array of microarrays. The device serves as a hybridization chamber for 49 different samples as well as the flow-cell during subsequent processing such as washing and staining. In this implementation, the assembly includes a two-piece frame, such as a top segment and a bottom segment holding the sample segment in place. Different modules can be attached on either side of the sample segment at different processing stages. During hybridization, the arrays and samples are kept separated from each other by a grid seal, such as a silicone seal held in place and pressed against the sample segment by a coated aluminum grid segment. A base segment, incorporating a bottom support such as solid aluminum plate attached to the frame provides support from the back to prevent breaking of the glass sample segment. Hybridization samples are applied to the arrays from above through the open grid. A volume of 300 µ L is sufficient to completely cover each array in this implementation. Evaporation is prevented by a solid lid pressing a second seal onto the grid plate (not shown). Following hybridization, the samples are recovered for possible reuse and wash buffer is added onto the arrays to prevent them from drying out.

[0086] In this implementation, to convert the hybridization chamber into a flow-cell for washing and staining of all the arrays in parallel, the grid plate is removed and a solid coated aluminum plate, held at a distance of 1.5 mm from the sample segment, is attached in its place. This creates a space between the oligonucleotides attached to the sample segment and the solid plate that can be filled and vented through two ports (inlet and outlet ports) in the frame. Removing the back support plate allows viewing of the sample segment and completes the conversion into a flow-cell with a total volume of about 35 ml. Two sets of washes of increasing stringency are performed after the hybridization to remove sample RNA non-specifically bound to the arrays. As in the standard procedure for Affymetrix ® GeneChip ® oligonucleotide microarrays, the entire sample segment is then stained with streptavidin-conjugated phycoerythrin, followed by further signal amplification with biotinylated anti-streptavidin and a second staining with streptavidin-conjugated phycoerythrin.

[0087] The described experimental approach greatly increases the rate at which, for example, gene expression profiles can be generated using microarrays and, continuing this example, facilitates the construction of large databases of gene expression patterns. Typically, both the sample preparation and whole sample segment hybridization may be accomplished by one person in little more time and effort than required to process a few individual samples and microarrays. Furthermore, many steps may be readily automatable and multiple plates and sample segments may be processed in parallel. Moreover, while sample segments with 7x7 individual microarrays were described with respect to this experimental implementation, sample segments with larger numbers of microarrays could be used. Also, as noted, the described apparatus and methods are generalizable and, as will now be appreciated by those of ordinary skill in the art in view of this disclosure, may be applied, for example, to cDNA arrays, SNP arrays, sequencing arrays, and tag arrays.

[0088] Multi-Array Scanning System 2700Figure 27 is a functional block diagram of illustrative multi-array scanning system 2700. System 2700 includes a convertible processing apparatus that is illustratively assumed to be apparatus 5 as described above with respect to Figures 1-26. It will be understood, however, that various other implementations of a convertible processing apparatus may be employed in other implementations. In other implementations, the convertible processing apparatus

includes one or more containing members constructed and arranged to contain the substrate. The apparatus also includes a separating member constructed and arranged so that, when the separating member is disposed in a first position with respect to the containing members, the at least two microarrays are fluidically separated from each other by the separating member, and, when the separating member is disposed in a second position with respect to the containing members, the at least two microarrays are fluidically coupled with each other.

[0089] Multi-array scanning system 2700 is constructed and arranged to scan arrays of microarrays disposed on a substrate. System 2700 includes a scanner apparatus constructed and arranged to detect emission signals from at least two of the plurality of microarrays, as represented by illustrative scanner apparatus 2760. Labeled targets in hybridized probe-target pairs in microarrays may be detected using any of a variety of commercial scanners. Scanners image the targets by, for example, detecting fluorescent or other emissions from the labels, or by detecting transmitted, reflected, or scattered radiation. These processes are generally and collectively referred to hereafter for convenience simply as involving the detection of emissions. Various detection schemes are employed depending on the type of emissions and other factors. A typical scheme employs optical and other elements to provide excitation light and to selectively collect the emissions. Also generally included are various light-detector systems employing photodiodes, charge-coupled devices, photomultiplier tubes, or similar devices to register the collected emissions. As noted, possible implementations of apparatus 2760 are described in U.S. Patent Nos. 5,981,956 and 6,207,960, incorporated by reference above. Other scanners or scanning systems are described in US Patent Nos. 5,143,854; 5,578,832; 5,631,734; 5,834,758; 6,025,601, and 6,252,236; in PCT Application PCT/US99/ 06097 (published as WO99/47964); and in U.S. Patent Application Serial No. 60/286,578, each of which is hereby incorporated herein by reference in its entirety for all purposes.

[0090] The scanning system provides data representing the intensities (and possibly other characteristics, such as color) of the detected emissions, as well as the locations on the substrate where the emissions were detected. The data typically are stored in a memory device in the form of a data file. One type of data file, sometimes referred to as an image file, typically includes intensity and location information corresponding to elemental sub-

areas of the scanned substrate. The term elemental in this context means that the intensities, and/or other characteristics, of the emissions from this area each are represented by a single value. When displayed as an image for viewing or processing, elemental picture elements, or pixels, often represent this information. Thus, for example, a pixel may have a single value representing the intensity of the elemental sub-area of the substrate from which the emissions were scanned. The pixel may also have another value representing another characteristic, such as color. For instance, a scanned elemental sub-area in which high-intensity emissions were detected may be represented by a pixel having high luminance (hereafter, a bright pixel), and low-intensity emissions may be represented by a pixel of low luminance (a dim pixel). Alternatively, the chromatic value of a pixel may be made to represent the intensity, color, or other characteristic of the detected emissions. Thus, an area of high-intensity emission may be displayed as a red pixel and an area of low-intensity emission as a blue pixel. As another example, detected emissions of one wavelength at a particular sub-area of the substrate may be represented as a red pixel, and emissions of a second wavelength detected at another sub-area may be represented by an adjacent blue pixel. Many other display schemes are known.

[0091] Generally, a human being may inspect a printed or displayed image constructed from the data in an image file and may identify those cells that are bright or dim, or are otherwise identified by a pixel characteristic (such as color). However, it frequently is desirable to provide this information in an automated, quantifiable, and repeatable way that is compatible with various image processing and/or analysis techniques. For example, the information may be provided to a computer that associates the locations where hybridized targets were detected with known locations where probes of known identities were synthesized or deposited. Information such as the nucleotide or monomer sequence of target DNA or RNA may then be deduced. Techniques for making these deductions are described, for example, in U.S. Patent No. 5,733,729 to Lipshutz, which hereby is incorporated by reference in its entirety for all purposes, and in U.S. Patent No. 5,837,832, noted and incorporated above. Among other purposes, the data may be used to study genetic characteristics and to detect mutations relevant to genetic and other diseases or conditions.

[0092] In the illustrated implementation of Figure 27, scanner apparatus 2760 includes an

excitation radiation source 2762 and a focusing system 2766 constructed and arranged to focus radiation from the excitation radiation source onto selected portions of the substrate of an array of microarrays such as array 2770. Focusing system 2766 may be an automatic focusing system. Array of microarrays 2770 could be disposed, for example, on glass plate 25. Also included in scanner apparatus 2760 is radiation direction system 2768 that is constructed and arranged to scan the focused excitation radiation across selected portions of the substrate. Another element of illustrative apparatus 2760 is detector 2764 that detects the emission signals from the substrate in response to the focused excitation radiation. Detector 2764 may, as in the illustrated implementation, provide to computer 2703 digital information representing detected emissions. For example, detector 2764 may provide image data file 2790 via an input device of input/output devices 2780. Various non-limiting implementations of elements 2766, 2768, 2762, and 2764 are described in U.S. Patent No. 6,207,960 and other patents describing scanner implementations cited above, all of which have been incorporated herein by reference. For example, as in the "960 patent, focusing system 2766 may include an objective lens having a ratio of scanning field diameter to focused spot diameter of greater than about 2000, and a numerical aperture greater than about 0.2. As another example of an implementation described in the "960 patent, radiation direction system 2768 may include a mirror selected from the group consisting of a galvanometric mirror, angularly oscillating mirror, or a rotating polyhedral mirror for reciprocally scanning the focused excitation radiation. In accordance with illustrative implementations described in that patent, the focused excitation radiation may be reciprocally scanned across a selected microarray of array of microarrays 2770 at a rate of at least 20 image lines per second.

[0093]

System 2700 of the illustrated implementation also includes a data acquisition system constructed and arranged to record an amount of the emission signals detected as a function of positions on the substrate from which the emission signals were emitted. The data acquisition system of the illustrated implementation includes a computer 2703 constructed and arranged to receive image data representing the detected emission signals from the scanner apparatus and to store the image data in the memory. Computer 2703 of this implementation includes processor 2705 and system memory 2720, as well as various other conventional computer components such as operating

system 2710, graphical user interface (GUI) controller 2715, memory storage devices 2725, input-output controllers 2730, and system bus 2704, the operations of which are familiar to those of ordinary skill in the relevant art. A scanner control, data acquisition, and data analysis application 2701 may be loaded via an input device of input/output devices 2701 into system memory 2720 or another memory unit so that, typically, it may be executed by processor 2705 in cooperation with operating system 2710, as represented by application executable 2701A. Application 2701 may be written in C, C++ or any of a variety of other programming languages.

[0094] When executing executable 2701A, computer 2703 controls the focusing system and the radiation direction system so as to sequentially focus on and irradiate a first of the microarrays in array 2770 and then sequentially focus on and irradiate one or more other microarrays in array 2770. In one implementation of this sequential operation, apparatus 5 is coupled (e.g., fasted by removable hinges, pins, adhesives, or other devices, or permanently secured in some implementations) to translation stage 2774. Although stage 2774 is shown in a horizontal position in Figure 27, it will be understood that any orientation generally is possible. Scanner apparatus 2760 of this implementation includes a translation stage controller 2763 that is constructed and arranged to move translation stage 2774, under the direction of computer 2703 executing executable 2701A, in coordination with focusing system 2766 and radiation direction system 2768. For example, translation stage controller 2763 may move translation stage 2774 in an X direction, a Y direction, or both so as to sequentially position various microarrays of array 2770 for irradiation by excitation radiation source 2762. In some implementations, the X and Y directions are orthogonal to each other. In these and other implementations, translation stage controller 2763 may move translation stage 2774 in a Z direction orthogonal to a plane of the X and Y directions so as to sequentially position each of the at least two microarrays for focusing.

[0095] Graphical user interfaces (GUI"s) 2782 may be presented to a user so that the user may select particular microarrays of array 2770, and their order, for scanning. For example, in accordance with any of a variety of conventional user-interface techniques, a user may be presented by GUI"s 2782 (under the control of GUI controller 2715) with a list of microarrays included in array 2770 identified, for example, by a list of names or identifiers, and/or by a graphical representation of the microarrays in an array

configuration on a monitor or other output device of input/output devices 2780. Thus, using the example given above in which array 2770 consists of 49 microarrays arranged in 7 rows and 7 columns, a graphic of 49 elements could be presented to the user. The user could select all 49 elements (using any of a variety of conventional user-interface selection techniques) if it were desired that each of the 49 microarrays were to be sequentially scanned. Alternatively, the user could select one or more of the 49 microarrays, in any designated order or combination, for sequential scanning. In yet another alternative implementation, a user could specify that two or more adjacent microarrays should be scanned together. That is, for example, focusing could be performed on microarray 1, and then microarray 1 and neighboring microarray 2 could be scanned simultaneously (e.g., using a wider scan than would be used for a single microarray so as to encompass both microarrays in a single scan line).

[0096] In a further implementation, detector 2764 may include, in addition to the optical and other components used to collect and detect emissions, a reader for detecting machine-readable code, e.g., a bar-code reader. Optionally, the reader may be a separate device included in apparatus 2760. In any of such implementations, a machine readable code, such as a bar code, may be imprinted on glass plate 25, or at another location on convertible processing apparatus 5 or translation stage 2774. This code may include, for example, a unique identifier for the array of microarrays or there may be multiple codes, optionally disposed within or in proximity to each of the microarrays, that identify each of the microarrays in array 2770. The code may also include information about or related to one or more of the microarrays. The code reader of detector 2764 or separate reader of apparatus 2760 may read the code and, in accordance with known techniques, provide it via an input device of input/output devices 2780 to executable 2701A. Executable 2701A may then, for example, compare a unique microarray identifier to a database (stored for instance in one of memory storage devices 2725, such as a hard drive) so that information linked to that identifier may be retrieved. This linked information may include, for example, parameters used to adjust one or more of the elements of scanner apparatus 2760 (e.g., to adjust excitation radiation source 2762 in view of a particular label used with the microarray identified by the identifier), or to adjust an algorithm used to analyze data in image data file 2790.

[0097] Having described various embodiments and implementations of the present

invention, it should be apparent to those skilled in the relevant art that the foregoing is illustrative only and not limiting, having been presented by way of example only. For example, many other schemes for distributing functions among the various elements of the illustrated embodiment are possible in accordance with the present invention. The functions of any element may be carried out in various ways in alternative embodiments. Also, the functions of several elements may, in alternative embodiments, be carried out by fewer, or a single, element. Numerous other embodiments, and modifications thereof, are contemplated as falling within the scope of the present invention as defined by appended claims and equivalents thereto.

[0098] What is claimed is: